

Amendments to the Claims

Claim 1. (Currently amended) A method for producing embryoid bodies (EBs) from pluripotent cells comprising

(i) obtaining a liquid single cell suspension culture of pluripotent cells;

(ii) collecting and suspending the cells in a container to a density of about 0.5×10^6 to 5×10^6 cells/ml;

(a)(iii) rocking [[a]] the container containing [[a]] the liquid single cell suspension culture ~~of pluripotent cells~~ thereby generating cell aggregates, ~~wherein said culture of pluripotent cells has a concentration of about 0.5×10^6 to 5×10^6 cells/ml; and~~

(b)(iv) diluting the suspension, and further rocking a container containing the suspension until formation of EBs; wherein the final concentration of EBs in the suspension culture is about 500 EBs/ml.

Claim 2. (Original) The method of claim 1, wherein prior to step (a) the cells are cultured on embryonic mouse fibroblasts (feeder cells).

Claim 3. (Previously presented) The method of claim 1 or 2, wherein said pluripotent cells are embryonic stem (ES) cells.

Claim 4. (Previously presented) The method of claim 3, wherein said cells are obtained from a murine ES cell line.

Claim 5. (Currently amended) The method of claim 1, wherein the culture medium in any or all of the steps step (a) or (b) or both is Iscove's Modified Dulbecco's

Media (IMDM), 20% fetal calf serum (FCS) and 5% CO₂.

Claim 6. (Currently amended) The method of claim 1, wherein the culture conditions in any or all of steps (i) through (iii) step (a) or (b) or both comprise 37°C and 95% humidity.

Claim 7. (Previously presented) The method of claim 1, wherein said culture of pluripotent cells has a concentration of about 1×10^6 to 5×10^6 cells/ml.

Claim 8. (Previously presented) The method of claim 1, wherein the suspension in step (a) is cultured for about 6 hours.

Claim 9. (Previously presented) The method of claim 8, wherein the suspension is cultured for about 16 to 20 hours.

Claim 10. (Previously presented) The method of any one of claims 1, 8 or 9, wherein the suspension in step (b) is cultured in T25 flasks.

Claim 11. (Previously presented) The method of claim 1, wherein said dilution in step (b) is 1:10.

Claim 12. (Canceled)

Claim 13. (Previously presented) The method of claim 1, further comprising

dividing the cell aggregates to the desired final concentration.

Claims 14-16. (Canceled)

Claim 17. (Previously presented) The method of claim 1, further comprising culturing the EBs under conditions allowing differentiation of the EBs into cardiomyocytes.

Claim 18. (Canceled)

Claim 19. (Previously presented) The method of claim 17, further comprising selection of cardiomyocytes by use of one or more selectable markers or agents or both.

Claim 20. (Previously presented) The method of claim 19, wherein said cell is genetically engineered.

Claim 21. (Previously presented) The method of claims 19 or 20, wherein said cell comprises a selectable marker or a reporter gene or both.

Claim 22. (Previously presented) The method of claim 21, wherein said cell comprises a selectable marker gene operably linked to a cell type-specific regulatory sequence.

Claim 23. (Original) The method of claim 22, wherein said selectable marker confers resistance to puromycin.

Claim 24. (Previously presented) The method of claim 21, wherein said cell comprises a reporter gene operably linked to a cell type-specific regulatory sequence.

Claim 25. (Previously presented) The method of claim 24, wherein said cell type-specific regulatory sequence of the reporter gene is the same as said cell type-specific regulatory sequence of the marker gene.

Claim 26. (Previously presented) The method of claim 25, wherein said reporter is enhanced green fluorescent protein (EGFP).

Claim 27. (Previously presented) The method of claim 21, wherein said marker gene and said reporter gene are contained on the same recombinant nucleic acid molecule.

Claim 28. (Original) The method of claim 27, wherein said marker gene and said reporter gene are contained on the same cistron.

Claim 29. (Previously presented) The method of claim 22, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.

Claim 30. (Previously presented) The method of claim 29, wherein said regulatory sequence is a cardiac-specific regulatory sequence selected from promoters of alpha-myosin heavy chain (alpha-MHC) or ventricular myosin light chain 2 (MLC2v).

Claim 31. (Previously presented) An embryoid body obtained by the method of claim 1.

Claim 32. (Previously presented) A cardiomyocyte or tissue of cardiomyocytes obtained from the embryoid body of claim 31.

Claims 33-44. (Canceled)

Claim 45. (Currently amended) A method for producing embryoid bodies (EBs) from pluripotent cells comprising

(i) obtaining a liquid single cell suspension culture of pluripotent cells;

(ii) collecting and suspending the cells in a container to a density of about 0.1×10^6 to 1×10^6 cells/ml;

(a)(iii) rocking [[a]] the container containing [[a]] the liquid single cell suspension culture of pluripotent cells thereby generating cell aggregates, wherein said culture of pluripotent cells has a concentration of about 0.1×10^6 to 1×10^6 cells/ml; and

(b)(iv) rocking [[a]] the container containing the suspension until formation of EBs; and

(v) diluting the resultant EBs to a concentration of about 100-2000 EBs/10 ml.

Claim 46. (Previously presented) The method of claim 45, wherein prior to step (a) the cells are cultured on embryonic mouse fibroblasts (feeder cells).

Claim 47. (Previously presented) The method of claim 45 or 46, wherein said pluripotent cells are embryonic stem (ES) cells.

Claim 48. (Previously presented) The method of claim 47, wherein said cells are obtained from a murine ES cell line.

Claim 49. (Currently amended) The method of claim 45, wherein the culture medium in any or all of the steps ~~step (a) or (b) or both~~ is Iscove's Modified Dulbecco's Media (IMDM), 20 % fetal calf serum (FCS) and 5 % CO₂.

Claim 50. (Currently amended) The method of claim 45, wherein the culture conditions in any or all of steps (i) through (iv) ~~step (a) or (b) or both~~ comprise 37°C and 95 % humidity.

Claim 51. (Previously presented) The method of claim 45, wherein said culture of pluripotent cells has a concentration of about 0.1×10^6 to 0.5×10^6 cells/ml.

Claim 52. (Previously presented) The method of claim 45, wherein the suspension is cultured for about 48 hours.

Claim 53. (Canceled)

Claim 54. (Previously presented) The method of claim 45, further comprising culturing the EBs under conditions allowing differentiation of the EBs into cardiomyocytes.

Claim 55. (Previously presented) The method of claim 54, further comprising selection of cardiomyocytes by use of one or more selectable markers or agents or both.

Claim 56. (Previously presented) The method of claim 55, wherein said cell is genetically engineered.

Claim 57. (Previously presented) The method of claims 55 or 56, wherein said cell comprises a selectable marker or a reporter gene or both.

Claim 58. (Previously presented) The method of claim 57, wherein said cell comprises a selectable marker gene operably linked to a cell type-specific regulatory sequence.

Claim 59. (Previously presented) The method of claim 58, wherein said selectable marker confers resistance to puromycin.

Claim 60. (Previously presented) The method of claim 57, wherein said cell comprises a reporter gene operably linked to a cell type-specific regulatory sequence.

Claim 61. (Previously presented) The method of claim 60, wherein said cell type-specific regulatory sequence of the reporter gene is the same as said cell type-specific regulatory sequence of the marker gene.

Claim 62. (Previously presented) The method of claim 61, wherein said reporter is enhanced green fluorescent protein (EGFP).

Claim 63. (Previously presented) The method of claim 57, wherein said marker gene and said reporter gene are contained on the same recombinant nucleic acid molecule.

Claim 64. (Previously presented) The method of claim 63, wherein said marker gene and said reporter gene are contained on the same cistron.

Claim 65. (Previously presented) The method of claim 58, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.

Claim 66. (Previously presented) The method of claim 65, wherein said regulatory sequence is a cardiac-specific regulatory sequence selected from promoters of alpha-myosin heavy chain (alpha-MHC) or ventricular myosin light chain 2 (MLC2v).

Claim 67. (Previously presented) An embryoid body obtained by the method of claim 45.

Claim 68. (Previously presented) A cardiomyocyte or tissue of cardiomyocytes obtained from the embryoid body of claim 67.

Claim 69. (Previously presented) The method of claim 24, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.

Claim 70. (Previously presented) The method of claim 60, wherein said cell type-specific regulatory sequence is atrial- or ventricular-specific.